

Echolocation and distribution of *Saccolaimus saccolaimus* in north-western Australia

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ABSTRACT – Specimens of the vulnerable bat *Saccolaimus saccolaimus* have recently been recognised among museum collections from north-western Australia. They include one whose search mode echolocation pulses were being recorded when it was collected. In terms of spectral characters, this pulse sequence was indistinguishable from reference sequences by *S. saccolaimus* from the Northern Territory.

We compare these reference sequences with free-flying sequences: (1) from the two other species at the same detection sites that have a similar acoustic niche (*Saccolaimus flaviventris* and *Chaerephon jobensis*), and (2) recorded from sites elsewhere in north-western Australia. Equivalent sequences were detected at 40 of 173 recording sites scattered throughout north-western Australia, and in all five of its bioregions, implying that *S. saccolaimus* is widespread at the western end of its geographical range in Australia. At an overall average frequency of 20.3 kHz, these sequences, like their Northern Territory counterparts, are significantly different from *S. flaviventris* and *C. jobensis* in all spectral parameters measured, and consistent with (albeit 2.8 kHz lower than) *S. saccolaimus* sequences from Queensland.

KEYWORDS: Kimberley, bats, museum collections, vulnerable species, Northern Territory, Queensland

INTRODUCTION

The vulnerable bat *Saccolaimus saccolaimus* (Temminck, 1838) is known in Australia from the high rainfall tropical regions of Northern Territory and Queensland (T.S.S.C. 2016), but data on its range limits and habitat associations are scant because there are few museum specimens and known localities. As Woinarski et al. (2014) pointed out, field surveys have been hampered by difficulties in diagnosing its echolocation sequences from those of co-occurring species such as *Saccolaimus flaviventris* (Peters, 1867) and *C. jobensis* (Miller, 1902). Historically, all Western Australian *Saccolaimus* have been identified as *S. flaviventris* because they lack the bare rump and/or numerous dorsal flecks, speckles or spots that characterise Queensland *S. saccolaimus*.

Two recent events prompted a reevaluation of these identifications. Firstly, a mitochondrial DNA study of specimens from Queensland and Northern Territory revealed that, although *S. saccolaimus* forms a single

genetic clade in Australia, a proportion of the Northern Territory *S. saccolaimus* population lacks the bare rump and/or dorsal markings (Milne et al. 2009). It also showed that adults have a narrower ‘outer-upper canine width’ (OC¹W < 5.7 mm) than *S. flaviventris*. Using this metric, specimens from five localities in the Kimberley, north-western Australia, were subsequently discovered in the Western Australian Museum (WAM) collection (see Woinarski et al. 2014): WAM M21831, M24019, M26078, M49194 and M64808 (Table 1). They did not have the distinctive bare rump, white dorsal flecks/speckles/spots, dark reddish-brown dorsal fur or pale brown to grey ventral fur of most individuals belonging to Queensland populations. Instead, they had uniform dark brown to black dorsal fur and creamy-white ventral fur just like *Saccolaimus flaviventris*, the only congeneric known from the Kimberley.

Secondly, during an acoustic survey of bats on islands along the Kimberley coast, we noticed that search mode echolocation sequences we were assigning to

S. flaviventris formed two discrete clusters in terms of spectral parameters. Subsequent study revealed that one of these clusters resembled *S. saccolaimus* sequences from the Northern Territory, illustrated in Milne et al. (2009). Fortuitously, the echolocation sequence emitted by one of the vouchered *S. saccolaimus* specimens was being recorded when it was shot (flying through the beam of a spotlight), thus providing a reference sequence from the Kimberley.

In this study we document the echolocation signature of *S. saccolaimus* and reassess its geographic range in the Kimberley, where it was virtually unknown. We provide comparison with free-flying reference echolocation sequences by its two acoustic neighbours (*S. flaviventris* and *C. jobensis*) to facilitate future surveys. Specifically, we assess the hypothesis that *S. saccolaimus* occurs throughout the Kimberley by (a) reappraising the *Saccolaimus* collections in the Western Australian Museum for adult specimens with 'outer-upper canine width' < 5.7 mm, and (b) by searching our

library of ultrasound recordings from the Kimberley for echolocation sequences that match the *S. saccolaimus* reference sequences from the Northern Territory and/or the Kimberley reference sequence. Animal Ethics Committee approvals: A02001 (NT) and DEC 2010/21 (WA).

MATERIALS AND METHODS

STUDY AREA

The Kimberley Administrative Region occupies north-western Australia and, for brevity, is referred to as the 'Kimberley' in this paper. Sparsely settled and with limited access, it spans 600 km north-to-south and 800 km east-to-west, includes both coastal and inland landscapes, and has a hot tropical climate. It is divided into five geomorphically distinctive bioregions (DSEWPac 2012), see Figure 1. The Northern and Central Kimberley bioregions comprise rugged uplands of the Kimberley Craton; the other three are

TABLE 1 Adult *S. saccolaimus* specimens from Kimberley locations (WAM# = Western Australian Museum specimen registration number).

^a Scaled from forearm length of the other female specimens.

^b Scaled from dried alcohol specimen using specimens of known fresh weight.

Site (Figure 1)	WAM#	Sex	Location (WMG)	Radius, D ³ P ¹ , OC'W (mm)	Weight (g)	Notes
10	M21831	♀	Mitchell Plateau at 14°49'16"S, 125°50'34"E	73.4, 31.1, 5.6		WAM/CRA on 25 April 1982
18	M49194	♂	Mitchell River track at 15°08'45"S, 126°09'30"E	73.3, 31.9, 5.7	38.2	Shot well above riparian canopy by N. McKenzie and J. Rolfe on 2 June 1987
128	M24045	♀	Tunnel Creek, near Fairfield HS. 17°36'26"S, 125°08'44"E	75.0, 30.8, 5.4	40.5 ^a	G. Barron & P. Griffin on 4 February 1982
152	M24019	♂	Barred Creek Bore. 17°39'45"S, 122°12'10"E	70.5, 30.9, 5.6		Shot above pindan canopy by G. Barron and P. Griffin on 24 January 1982
A	M26078	♂	Sale River at 16°01'37"S, 124°45'52"E	71.2, 30.5, 5.6	39.1	Shot in open air above riparian canopy by K.F. Kenneally on 13 May 1986
B	M64808	♀	Kimbolton HS. 16°41'00"S, 123°50'00"E	73.3, 31.1, 5.5	40.8 ^b	K. Goodchild in 2006
C	M22535	♀	Coulomb Point. 17°20'10"S, 122°09'40"E	--, 33.3, 5.7	45.0	Shot 15 m above shrubland on beach dune by N. McKenzie on 19 April 1987
D	M44078	-	Osmond Range 17°16'35"S, 128°15'30"E	75.9, 31.2, 5.6		S. Eberhard on 7 August 1998

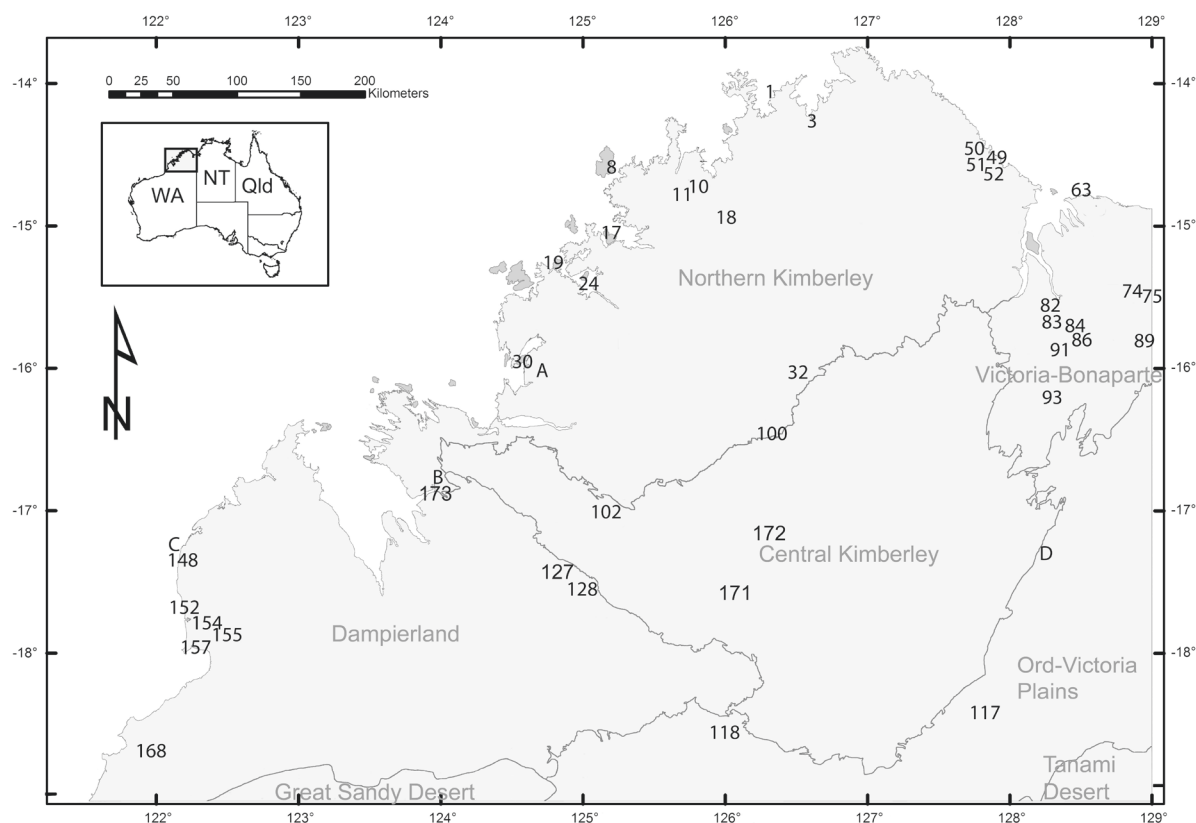


FIGURE 1 The Kimberley, showing the boundaries of its five biogeographic regions ('bioregions'), and the 40 sites where *S. saccolaimus* sequences were detected (Appendix 4). Museum voucher specimens exist from four of these 'numbered' sites as well as four others (marked as A to D in Table 1). Relevant Australian States are labelled: WA (Western Australia), NT (Northern Territory) and Qld (Queensland).

in sedimentary basins on its eastern, south-eastern and southern sides. The most mesic, the Northern Kimberley, encompasses 82,540 km² of sub-humid near-coastal as well as inland landscapes. The most arid, Dampierland, comprises 88,120 km² of semi-arid near-coastal and inland landscapes, mostly plains, and forms the north-western margin of the Canning Basin. The rest of the Canning Basin is occupied by the Great Sandy Desert.

Average annual rainfall ranges from 1,500 mm in the north-west to 400 mm in the south where it is locally unreliable, while average temperatures range from a daily maximum of 32.8°C in January to a night time minimum of 12°C in July. Nearly all rain falls during the 4 to 5 months of the 'wet' (November to March), the season of high humidity. The Kimberley Craton is drained by seasonally active river systems fringed by tall trees that form riparian forests or woodlands, and permanent pools are common. Its landscapes support savannas, tree steppes, woodlands and open forests. They include undulating hill country with broad valleys and rugged boulder country associated with extensive basalt, quartz sandstone and limestone karst uplands, ranges and plateaus dissected by gorges.

Two compositionally distinct zoophagic bat ensembles occur in the Kimberley. One occupies landward environments and comprises 19 species; the other occupies mangroves and comprises 16 species. Their deterministic compositional structure has been displayed in terms of acoustic parameters, foraging strategy and foraging microhabitat (McKenzie and Bullen 2012: Fig. 4).

EXISTING SPECIMENS FROM THE KIMBERLEY

We reexamined the WA Museum's *Saccolaimus* collection in search of adults with an 'outside canine width' of less than 5.7 mm. We also recorded dorsal and ventral fur for overall colour, flecks, speckles or spots, and checked for bare areas on the rump.

ACOUSTIC SURVEY

Between 1987 and 2017, we sampled 173 sites scattered across the Kimberley using bat detectors placed at ground-level with microphones orientated near-vertically upwards to minimise echoes. The recording equipment improved during the sampling program. For the first 73 sites we used an Anabat II bat detector coupled to a Sony Walkman WMD6C cassette recorder or, after 2003, a Sony Walkman mono minidisc recorder

(128 kHz sampling rate) or, after 2010, an Olympus LS10 digital recorder (see McKenzie et al. 2012). In all cases the detector's output was saved in pulse code modulation (PCM) wave format. From 2012 onwards, we used a Wildlife Acoustics SM2BAT full-spectrum ultrasound recorder (384 kHz sampling rate) coupled with an omni-directional SMX-US ultrasonic microphone to sample another 100 sites. Taped to the tip of a stake, the microphone was about 1.5 m above the ground and several metres from obstructions. The SM2 recorder was pre-programmed to switch on at late dusk and record until dawn. Kaleidoscope (Version 2.3.0, Wildlife Acoustics 2015) was used to extract bat echolocation sequences from the SM2 recordings, and save them as individual sound files in PCM wave format. Sites were sampled for at least one night each with the SM2, but at least two nights each with the Anabat systems, supplemented with mist nets and/or spotlight collecting at 78 of the (mostly) pre-2012 sites (Animal Ethics approvals: DEC AEC 2007/03, 2010/21).

Rather than randomly arrayed, the recording sites were strategically selected to sample the study area's main ecosystems and provide geographical coverage. Most were in or within 0.2 km of savanna woodlands (146 sites), rainforest (26), mangroves (47), and/or riparian zones (69). They were in, or within 0.2 km of, plains country (59), undulating hill country (62) and/or rugged range country with crevices and caves (71).

COOL EDIT 2000, now 'Adobe Audition 2' (Adobe Systems, USA), was used to display each echolocation sequence in spectral view and measure pulse parameters such as peak frequency (F_{peak}) and fineness-of-tuning (Q), as described in McKenzie and Bullen (2012). Q is the width of the frequency band 6 dB below peak, taken from the Fast Fourier Transform (i.e. $Q_{-6\text{dB}}$). Q -values were corrected to allow for the higher resolution offered

by the SM2's full-spectrum recordings compared to the frequency-divided (/16) output from Anabat detectors, then standardised at $Q_{\text{SM2}}/3.0$ for calculation purposes. The correction factor was derived empirically using field-recorded sequences emitted by species whose search mode pulse frequencies straddle the relevant 17 to 24 kHz frequency band, i.e. *S. flaviventris* and *Taphozous georgianus* (Appendix 1).

Sequences were identified to species using the reference echolocation library and procedure described in McKenzie and Bullen (2012). This library comprises PCM wave file recordings of known free-flying individuals (cylume-tagged or shot). It comprises 26 species (all species known from the Kimberley) and 248 sequences with an average of 9.5 sequences per species (s.d. = 6), and 2,322 individual pulses at an average of 89 pulses per species (s.d. = 46). Thus, F_{peak} and Q values were determined for several of the search-mode pulses comprising a sequence, along with pulse repetition-rate (to confirm that the bat was in search mode), shape, duration (Dur), and overall slope ($(F_{\text{max}} - F_{\text{characteristic}})/\text{duration}$). F_{peak} is equivalent to F_{peakC} when Anabat wave files were used, and to ' $0.94 F_{\text{characteristic}} + 1.57$ ' ($R^2 = 0.95$, $n = 18$) when Anabat-ZCAIM files (see Corben 2004) were used, i.e. for the three Northern Territory reference sequences described below. Because its component variables are independent of the hardware, the *Slope* equation should yield comparable values irrespective of whether recordings are processed using Anabat-ZCAIM or SM2-wave file formats, but we are yet to derive an algorithm yielding reliable *Slope* values from Anabat wave files.

The echolocation library comprises a scatterplot of pulse F_{peakC} versus Q , as well as a table summarising other pulse and sequence attribute values for species in free-flight, such as pulse duration and repetition rate.

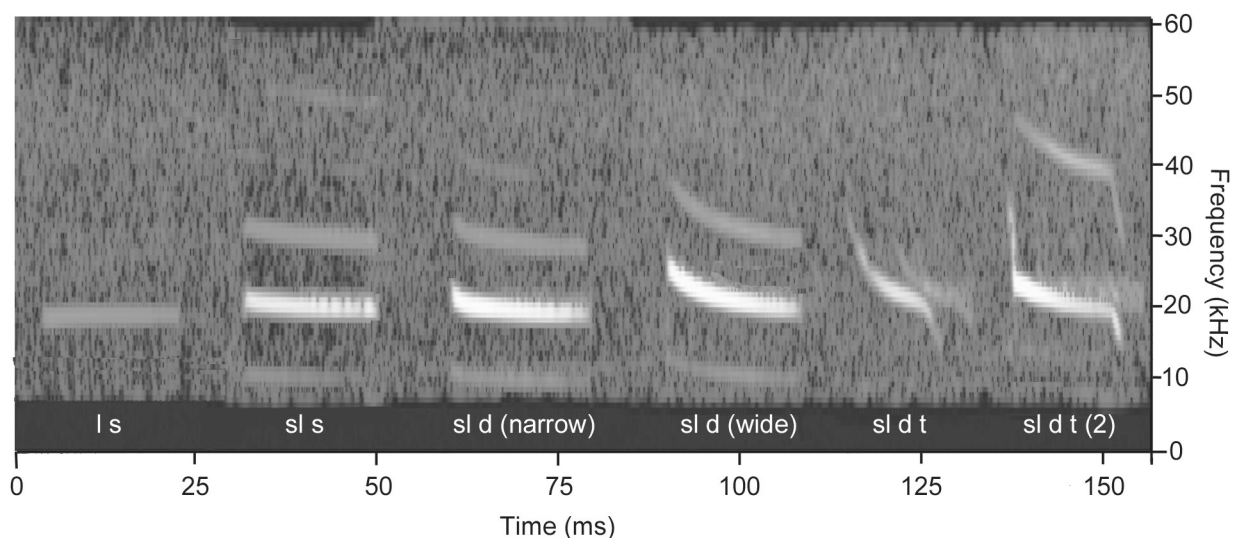


FIGURE 2 Spectral view of typical search-mode pulses by Kimberley *S. saccolaimus*, displaying different search mode pulse shapes. From left to right: level straight ('l s', F_{peak} 18.7 kHz, duration 19 ms, *Slope* 0.00 kHz ms⁻¹), slant straight ('sl s', 19.9, 16, 0.08), narrow bandwidth slant dished ('sl d', 19.4, 18, 0.12), wide bandwidth slant dished ('sl d', 20.7, 15, 0.29), slant dished with tail ('sl d t', 21.3, 15, 0.51), and a second version of 'sl d t' (21.1, 14, 0.28).

In diagnosing pulse sequences, we ignored pulses that did not meet the clarity, duration, shape and sequence repetition rate criteria used in compiling the library. For instance, the echo that partially overlies pulse ‘sl d t’ in Figure 2 can induce errors such as low Q estimates and/or exaggerated duration values. Unknown pulses that plotted on the periphery of a pulse cluster in the library scatterplot were identified only if they were in continuous sequence with pulses that fell within the cluster. Unknown pulses that plotted in an area of overlap between two species were not identified unless they were in continuous sequence with at least three pulses that fell in an area of the cluster that was outside of the overlap.

S. saccolaimus was not included in our 2012 echolocation library for the Kimberley. At that time, search mode echolocation sequences were assigned to *S. flaviventris* if they included undistorted pulses at $14 < F_{\text{peakC}} < 21$ kHz with $Q > 11$ (strongest harmonic ‘frequency at peak cycles’ and ‘finesse-of-tuning’, respectively, from Anabat recordings) that were > 17 ms long and emitted at an integer fraction of *circa* 7 Hz (McKenzie and Bullen 2012: i.e. c. 3.7 Hz if emitted every second wing beat, c. 2.3 Hz if every third, c. 1.75 Hz if every fourth, etc). Oddly, some sequences were comprised entirely of pulses at > 19 kHz. Also, these pulses had unexpectedly high Q -values, given the inverse relationship between F_{peakC} and Q shown by emballonurid bats (McKenzie and Bullen 2012). In addition, the detectable bandwidth of the strongest harmonic in these odd pulses was surprisingly narrow for field recordings of free-flying *S. flaviventris* near the high end of its search mode frequency range, and resembled the pulses in two of the Northern Territory *S. saccolaimus* sequences illustrated by Milne et al. (2009: Figs 4b–c).

Consequently, we have reassigned Kimberley sequences to *S. saccolaimus* if they match, or are in continuous sequence with pulse-series that match, (1) the Kimberley reference sequence (by specimen M49194, flying in open air above tree canopy) and/or (2) the descriptors for sequence-types (b) and (c) in Fig. 4 of Milne et al. (2009). Their study identified three different search mode sequences from Anabat-ZCAIM reference recordings of Northern Territory *S. saccolaimus*, each emitted in a different environmental setting. Within each sequence-type, the individual pulses were of similar shape. Three distinct shapes can be discerned, and are among those illustrated in Figure 2:

1. Narrow bandwidth slant dished (‘sl d’) pulses, emitted in open air very high above tree canopy (Milne’s type (b), Northern Territory Museum specimen U4080),
2. a wide bandwidth version of ‘sl d’, emitted ‘just above the tree canopy’ (Milne’s type (c), NTM U5231), and
3. slant dished with a tail (‘sl d t’) pulses, emitted ‘*2m above the ground*’ (Milne’s type (a), NTM U4079).

In terms of ‘minimum frequency and characteristic call frequency’, Milne et al. (2009) concluded that all three types were different from pulse sequences by its syntopic acoustic neighbour, *S. flaviventris*, but having lumped the three types together for the statistical comparison, found ‘...the call signature could not be reliably used to distinguish’ these two species (p. 506).

Using ‘species+pulse shape’ as the classification variable (see below), Canonical Discriminant Analysis (STATISTICA version 6) was used to test whether *S. flaviventris*, *S. saccolaimus* and *C. jobensis* echolocation sequences could be separated in terms of the pulse sequence attributes listed earlier. The data points were the averaged ultrasound attributes of each sequence in the reference library, the type (b) and (c) reference sequences from the Northern Territory, and the Kimberley sequences that we assigned to *S. saccolaimus*. Sequences averaging > 21.1 kHz were excluded to avoid approach-mode pulses. Because we do not have an algorithm yielding reliable *Slope* values from Anabat wave files, the 98 data points (57%) derived from these recordings could not be included in the discriminant function analysis.

RESULTS

EXISTING KIMBERLEY SPECIMENS

Our search of the WA Museum collection revealed three additional specimens of *S. saccolaimus* from Kimberley localities (M44078, M24045 and M22535), making a total of eight (Table 1), all collected between 1977 and 2006. Three of the eight specimens came from sites that we subsequently sampled using ultrasound detectors and, fortuitously, one (M49194) was shot while its echolocation pulses were being recorded.

In terms of adult forearm length and body weight, available Kimberley specimens are within the ranges reported for Northern Territory specimens: 40.7 g (S.D. = 2.6, N = 5) and 73.2 mm (1.9, 7) from Table 1, compared to 47.2 g (11.6, 3) and 74.9 mm (2.1, 22) from Milne et al. (2009), respectively. Also consistent with Northern Territory specimens, none of the eight Kimberley specimens have naked rumps, and the fur on their dorsal surfaces is very dark brown. Two have white hairs in one to four diffuse, mid-dorsal patches (M26078 and M22535). M26078 also has a few individual white hairs elsewhere on its back, and M49194 has just two such hairs but no patches. Ventral surfaces are covered with uniform white or cream fur in all eight cases.

ECHOLOCATION DETECTIONS IN THE KIMBERLEY

Pulses comprising the Northern Territory and Kimberley reference sequences that were recorded from *S. saccolaimus* flying in open air space (‘above the tree canopy’) did not show significant differences in F_{peakC} , Shape, *Slope* or *Dur* (Table 2). Two additional pulse shapes illustrated in Figure 2 (‘1 s’ and ‘sl s’) were accepted as *S. saccolaimus* because they were

in-sequence with pulse shapes present in the Northern Territory and/or Kimberley reference sequences ('sl d' and 'sl d t').

The averaged spectral statistics of the search mode echolocation sequences used as data points for the discriminant function analysis (DFA) are displayed in Table 3. Shape (Figure 2) was used as the grouping variable. Because the frequency-, *Slope*- and *Q*-values of sequences comprising pulse shapes 'l s' and 'sl s', as well as 'sl d' and 'sl d t', were not statistically separable for either *C. jobensis* or *S. saccolaimus* (i.e. the standard deviations overlapped the averages), these shape categories were merged in the DFA. Furthermore,

since only a single search mode sequence comprising 'ss t' pulses was observed (by *C. jobensis*), it could not be included in the analysis as a separate group, so was added to *C. jobensis*'s 'sl d t + sl d' group for the analysis. Thus, we pooled the *C. jobensis* sequences into two 'observed' groups ('l s + sl s' and 'sl d + sl d t + ss t'), *S. saccolaimus* sequences into two 'observed' groups ('l s' + 'sl s' and 'sl d + sl d t'), but left *S. flaviventris* sequences as three groups ('l s', 'sl s' and 'sl d').

Discriminant analysis of these sequences showed that the three bat species were distinct in terms of the spectral attributes assessed. The first discriminant function [$F_n(1) = -1.35F_{\text{peak}} + 0.35\text{Slope} - 1.38Q + 0.51\text{Dur}$]

TABLE 2 Comparison of echolocation pulses comprising reference sequences recorded from Northern Territory (NTM U4080) and Kimberley (WAM49194) *S. saccolaimus*: Average (s.d., number of pulses); d.f. = 16, 2-tailed test.

	F_{peak} (kHz)	<i>Slope</i> (kHz ms ⁻¹)	<i>Dur</i> (ms)	Shape
M49194	20.7 (0.2, 9)	0.08 (0.03, 9)	15.1 (0.8, 9)	sl d
U4080	21.0 (0.5, 9)	0.09 (0.02, 9)	15.1 (0.7, 9)	sl d
Student- <i>t</i> (p.)	-1.55 (0.14)	-0.70 (0.49)	0.00 (1.00)	

TABLE 3 Averaged spectral statistics of the strongest harmonic in search mode echolocation sequences: Mean (s.d.) of sequences in terms of the peak frequencies (av*F*), slopes (av*Slope*), av*Q* and durations (av*Dur*) of their component pulses. *Chaerephon jobensis* (*Cj*), *S. saccolaimus* (*Sc*), *S. flaviventris* (*Sf*). Pulse shape categories: level straight (l s), slant straight (sl s), slant dish (sl d), slant dish with tail (sl d t) and s-shaped with tail (ss t). Total numbers, mean (s.d.) of pulses measured in each shape category are also provided, along with number of sequences and different localities represented. Kimberley bioregions are Dampierland (DL), Northern Kimberley (NK), Central Kimberley (CK), Ord-Victoria Plains (OVP) and Victoria-Bonaparte (VB). Northern Territory (NT) bioregions are Gulf Coast (GUC) and Top End Coast (TEC).

	Shape	av <i>F</i>	av <i>Slope</i>	av <i>Q</i>	av <i>Dur</i>	Seq. Pulses	Localities	Bioregions
<i>Cj</i>	ls	14.7 (0.6)	0.02 (0.01)	11.1 (0.1)	16.2 (0.2)	2 4	2	NK, CK
	sl s	14.6 (0.6)	0.03 (0.01)	11.4 (0.9)	15.3 (0.8)	3 13, 4.3 (2.1)	3	NK, DL
	sl d	18.4 (1.8)	0.43 (0.18)	8.4 (1.8)	13.0 (1.9)	7 20, 2.9 (2.6)	4	NK, VB, CK, DL
	sl d t	17.9 (1.5)	0.36 (0.16)	9.9 (1.2)	14.6 (1.3)	13 100, 7.7 (5.4)	6	NK, VB, CK, DL
	ss t	20.7	0.95	6.2	10.0	1 9	1	VB
<i>Sc</i>	ls	19.6 (0.5)	0.05 (0.02)	16.1 (0.3)	18.1 (0.7)	3 22, 7.3 (3.2)	2	NK, DL
	sl s	20.0 (0.7)	0.14 (0.08)	15.1 (1.2)	17.4 (3.1)	5 16, 3.2 (3.3)	4	NK, DL
	sl d	20.4 (0.4)	0.28 (0.09)	12.9 (1.2)	15.0 (2.6)	16 83, 5.2 (4.1)	13	NK, VB, CK, DL and GUC. TEC in the NT
	sl d t	20.4 (0.5)	0.31 (0.04)	13.0 (1.1)	14.3 (1.2)	4 9, 2.3 (1.9)	3	NK, VB, DL
<i>Sf</i>	ls	16.7 (1.0)	0.05 (0.02)	13.0 (0.3)	17.8 (1.0)	6 23, 3.8 (4.6)	4	NK, VB, CK, DL
	sl s	17.7 (1.0)	0.10 (0.02)	13.1 (0.8)	18.5 (1.8)	4 16, 4.0 (2.4)	3	NK, VB, DL
	sl d	19.6 (1.1)	0.33 (0.16)	11.5 (2.2)	14.2 (4.2)	13 40, 3.1 (2.2)	11	NK, CK, OVP, DL

TABLE 4 Pulse fineness-of-tuning (Q) statistics according to F_{peak} . Comprises Q_{Anabat} and $Q_{\text{SM2/3.0}}$ values. Average Q (s.d.), 'rn' = number of reference pulses, 'rN' = number of reference sequences, 'n' = number of pulses, 'N' = number of sequences, 'p/s' = average number of pulses per sequence (s.d.).

	F_{peak} (Khz)	<16	16	17	18	19	20	21	22	Totals
	Q	11.1 (1.0)	11.4 (1.5)	10.5 (1.8)	9.4 (1.9)	8.7 (1.6)	7.1 (1.4)	7.1 (1.3)		
<i>Cj</i>	rn, rN	37, 9	36, 12	59, 20	74, 22	59, 22	43, 18	10, 8		318, 42
	p/s									7.6 (6.0)
	Q	13.6 (1.2)	13.1 (0.9)	13.5 (1.2)	13.6 (1.8)	12.2 (2.5)	9.4 (1.2)	8.9 (1.8)		
<i>Sf</i>	rn, rN	11, 8	51, 10	66, 17	55, 17	59, 16	29, 13	4, 4		275, 48
	p/s									5.8 (6.7)
	Q				16.4 (1.9)	14.8 (1.6)	13.1 (1.9)	11.2 (2.1)	8.3 (1.4)	
<i>Sc</i>	n, N				6, 2	93, 15	91, 21	51, 10	13, 3	254, 36
	rn, rN				0, 0	4, 1	20, 3	13, 2	0, 0	37, 3
	p/s									6.4 (6.6)

had a canonical correlation coefficient of 0.96 and accounted for 90% of the observed variance in the echolocation data ($p < 0.00001$). The second function [$\text{Fn}(1) = -0.18F_{\text{peak}} + 0.85\text{Slope} - 1.06Q + 0.08\text{Dur}$] had a canonical correlation coefficient of 0.71, accounted for 9% of the remaining variation, and was also significant ($p < 0.00001$). The coefficients in these functions have been standardised; they reveal the relative contributions of the spectral attributes in separating species and their shape groups.

Unstandardised coefficients were used to generate the discriminant scatterplot (Figure 3). Only one of the 77 sequences was classified to the wrong species (posterior probability assignments are tabulated in Appendix 2). An 'assigned' sequence of five 14 ms 'slant-dished' pulses (Sc2 125), it was originally assigned to *S. saccolaimus*, but classified to *S. flaviventris* (posterior probability = 0.62) rather than *S. saccolaimus* (0.37) because its average Q of 10.9 was slightly low for *S. saccolaimus* at 20.6 kHz (see Table 4, above). The three reference *S. saccolaimus* sequences were correctly classified: M49194 (ScrK, posterior probability 0.91), U4080 (ScrNToc, 0.92) and U5231 (ScrNTac, 0.73). We conclude that Kimberley *S. saccolaimus* sequences are consistent with their Northern Territory counterparts but distinct from *S. flaviventris* and *C. jobensis*.

The coefficients of the standardised discriminant functions listed above reveal that two of the four variables contributed most to species separation (F_{peak} and Q). To widen the comparison, characterise the differences and simplify species recognition, we tabulated these two variables for the individual search mode echolocation pulses comprising (1) *S. flaviventris* and *C. jobensis* reference sequences from the Kimberley and elsewhere in tropical Western Australia, (2)

the Kimberley sequences that were classified as *S. saccolaimus* by the DFA, and (3) *S. saccolaimus* reference sequences from the Northern Territory and Kimberley (Table 4).

The differences in Q were significant ($p < 0.05$) because the standard deviation did not overlap the mean of another species in any frequency band (Table 4). In search-mode, *S. saccolaimus* uses the upper half of the frequency band occupied by syntopic *C. jobensis* and *S. flaviventris* (Table 4), but at these frequencies (between 18.7 and 21.1 kHz), *S. saccolaimus* pulses are more finely tuned (i.e. have higher Q , Student- $t = -4.620$, $p < 0.0001$, d.f. = 39, 2-tailed test) and have lower *Slope* values ($t = -3.709$, $p < 0.0001$, 31 (Table 3)). Appendix 3 provides relevant statistics for the pulse-shape categories of Kimberley *S. saccolaimus*, *S. flaviventris* and *C. jobensis*.

With an average F_{peak} of 23.1 kHz, *S. saccolaimus* sequences from a Queensland population (Table 5) were 2.8 kHz higher than their Northern Territory/Kimberley counterparts, but consistent in all other characters assessed.

Overall, echolocation sequences referable to *S. saccolaimus* were recorded at 40 (Figure 1, Appendix 4) of the 173 Kimberley sites where ultrasound recorders were deployed. The recordings confirm that it occurs throughout all five Kimberley bioregions (Table 3), and forages over all major ecosystems, both sub-humid and semi-arid. Sequences were recorded from tropical woodlands and savannas on rugged sandstone and limestone uplands, ranges and gorges, as well as Carson Volcanics hill country, paperbark woodland associated with coastal sand dunes, mangroves, pindan on sandplains, and riparian forests.

TABLE 5

Search-mode echolocation sequence statistics of Queensland (Qld) *S. saccolaimus* (Sc) derived from the strongest harmonic of free-flying pulse sequences (mean (s.d.)). The first column is derived from faint background sequences in the Cairns roost recordings supplied by G. Ford – probably individuals flying in more open airspaces above the trees. The second column is from individuals recorded as they flew towards the microphone after emerging from a roost. These sequences were recorded using a Wildlife Acoustics EM-Touch ‘full wave’ ultrasound recorder at a sampling rate of 250 kHz. Parameters are explained in Table 3. ‘Shape’ refers to the pulse’s appearance in spectral view (see Figure 2).

	Qld Sc	Qld Sc	Total
av F_{peak} range	21.3 – 22.8	23.8 – 24.7	
av F_{peak} (kHz)	22.1 (0.4)	24.2 (0.3)	23.1 (1.1)
avDur (ms)	17.9 (1.1)	14.1 (1.4)	16.1 (2.4)
avSlope (kHz ms ⁻¹)	0.07 (0.01)	0.12 (0.01)	0.09 (0.03)
Shape	sl s, sl d, sl d t	sl d, sl d t	
locations	1	1	1
sequences	9	8	17
pulses	31	52	83
pulses per sequence	5.3 (2.5)	4.7 (2.1)	

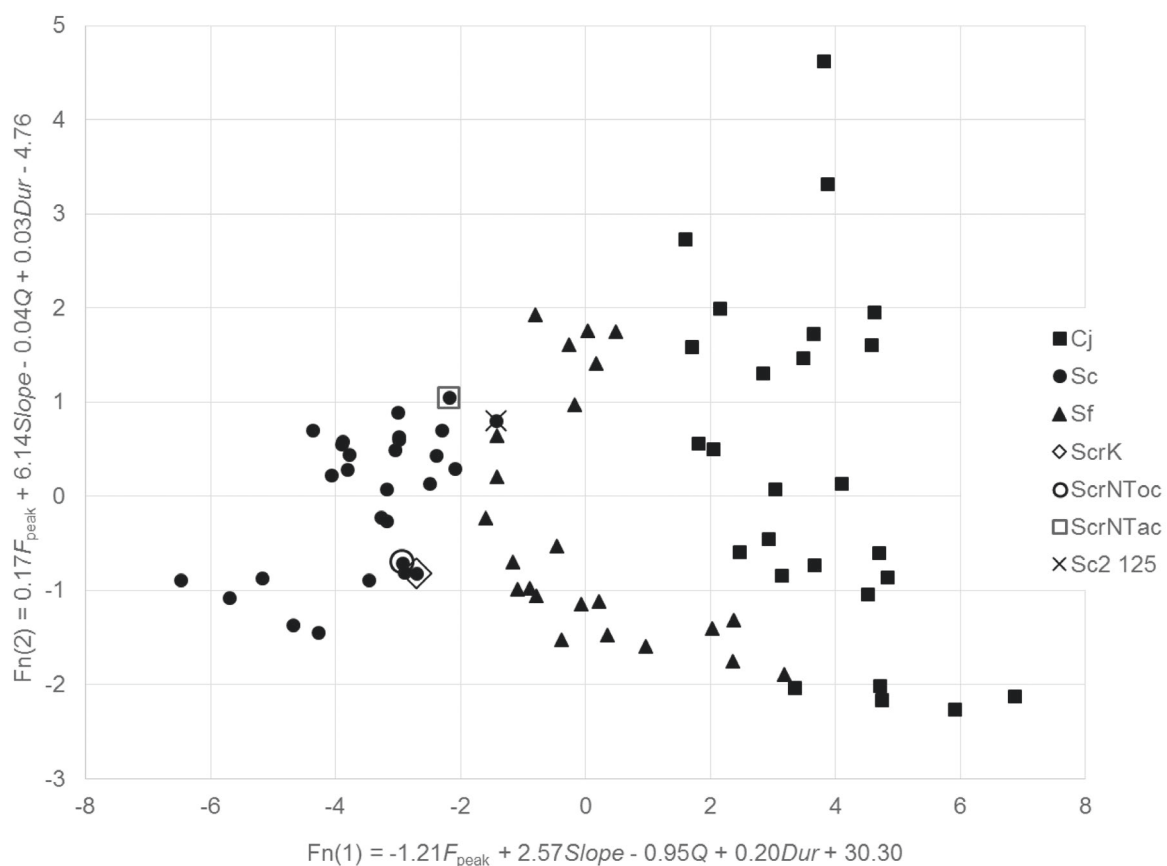


FIGURE 3

Standard DFA scatterplot of unstandardised canonical coordinates for sequences. Fn(1) Chi-square = 230.6 (d.f. = 24, p. < 0.00000), and Fn(2) Chi-square = 59.1 (15, <0.00000). *Cj* (*C. jobensis*), *Sc* (*S. saccolaimus*) and *Sf* (*S. flaviventris*). The three reference *S. saccolaimus* sequences are circled and the misclassified sequence point is crossed.

DISCUSSION

Voucher specimens in Western Australian Museum collections confirm that two species of *Saccolaimus* were extant in the Kimberley at least until 2006: *S. flaviventris* and *S. saccolaimus*. Like their Northern Territory counterparts, the two Kimberley *Saccolaimus* are so similar externally that zoologists had treated them as a single species. A DNA-verified *S. saccolaimus* specimen was collected at 15°45'S, 129°10'E (Milne et al. 2009), only 20 km north-east of our detection site 89 (Figure 1). Given its mode flight speed is c. 8 m/s (26 km/h) and the continuity in appropriate habitat, we surmise that the Kimberley population is continuous with the Northern Territory population. The eight *S. saccolaimus* specimens were collected from landward environments of three Kimberley bioregions: Northern Kimberley (4 locations), Dampierland (3) and Ord-Victoria Plains (1).

Our analysis of ultrasound recordings from the Kimberley confirm that both species persist there today. For pulses of the same shape ('sl d'), the search-mode parameters of the vouchered central Northern Territory and western Kimberley sequences showed no significant differences. In fact, most Kimberley sequences matched one of the three 'flight-space specific' search-mode sequence-types documented for Northern Territory *S. saccolaimus* by Milne et al. (2009, p. 504), and the discriminant function analysis classified these Northern Territory sequences to the same species as the Kimberley *S. saccolaimus* sequences (Figure 3). Furthermore, these Northern Territory and Kimberley sequences showed the same narrow bandwidth (3.5 kHz, i.e. 18.7–22.2), in contrast to the wider bandwidth of *S. flaviventris* (6 kHz, i.e. 15–21) (Table 4).

The third Northern Territory reference sequence also showed a similar pulse shape ('sl d t') to some Kimberley sequences, although its pulses were shorter in duration (10 vs 13 ms), and higher in frequency (22.9 vs 21.1 kHz), so we cannot exclude the possibility that this Northern Territory sequence is in transition to approach mode. Even so, in terms of these two spectral variables, this Northern Territory sequence is within two standard deviations of comparable sequences from the Kimberley (Appendix 3).

In combination, our detection of *S. saccolaimus* at 40 of the 173 sites sampled in the Kimberley, in all five Kimberley bioregions, and in both landward and mangrove communities (Figure 1 and Appendix 4), suggests that this species is widespread at the western end of its Australian range. We detected *S. saccolaimus* foraging over all major ecosystems in the Kimberley, both sub-humid and semi-arid. Its habitats in the Northern Territory and Queensland are known to be similarly diverse, including *Eucalyptus miniata*-*E. tetradonta* woodland (Milne et al. 2009) as well as mangroves, lowland tropical rainforest and littoral forest (Meutstege et al. 2014).

The echolocation signatures identified in this study will provide a quick, unambiguous basis for field survey of *S. saccolaimus*, without disrupting the normal activities of this vulnerable bat.

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ELECTRONIC APPENDICES

- 1 Comparison of Q_{6dB} values derived from Anabat and SM2BAT+ recordings of search mode echolocation sequences by *S. flaviventris* and *T. georgianus*.
- 2 Posterior probability matrix derived from the discriminant function analysis.
- 3 Statistics of the strongest harmonic in search-mode echolocation pulses according to shape.
- 4 Site location, habitat and bioclimatic attributes.

An electronic version of these appendices are available to download here:
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